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Applicant : Cindie M. Luhman

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ENHANCING MILK COMPONENT
CONCENTRATIONS

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Examiner: N. Levy

#24

SECOND DECLARATION UNDER 35 U.S.C. §132

Commissioner of Patents and Trademarks
Washington, D.C. 20231

SENT VIA EXPRESS MAIL

Express Mail No.: EV168042514 US

Sir:

I, Paul A. Porter, of 1025 190th Street, Webster City, Iowa, hereby declare as follows:

1. I am currently the Dairy Research Manager of the Land O' Lakes Research Farm in Webster City, Iowa.
2. I obtained a Bachelor of Science (B.S.) degree in Chemistry from Wittenberg University of Springfield, Ohio in 1981.
3. I obtained a Masters of Science (M.S.) in Animal Science from Cornell University in Ithaca, New York in 1984.
4. I obtained a Doctorate of Philosophy (Ph.D.) in Animal Science, with an emphasis in Dairy Nutrition, from Cornell University in Ithaca, New York in 1987.
5. Since completing my Doctorate of Philosophy degree in 1987, I have continuously been employed in positions directly relating to dairy herd management and nutrition.

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6. From 1987 to 1989, I was an Assistant Professor in the Department of Animal Science at Oregon State University in Corvallis, Oregon, where I conducted applied dairy nutrition research; taught courses in ration balancing, dairy herd management, and advanced dairy herd management; and served as a liaison to the Oregon State Dairy Industry.
7. From 1989 to 1992, I was the Dairy Program Manager for Countrymark Co-op of Delaware, Ohio, where I provided technical assistance, troubleshooting expertise, product support, and training to local dairy cooperatives, sales staff of the local dairy cooperatives, and dairy farm clients of the local dairy cooperatives.
8. From 1992 to 1994, I provided dairy sales and dairy consulting services for Young's Livestock Nutritional Services of Canastota, New York, where I analyzed dairy production records, consulted on dairy production issues, and designed customized dairy herd feeding programs for dairy farm clients.
9. From 1994 to 2000, I was Dairy Nutritionist and Field Technical Services Manager for Land O' Lakes Dairy Feed of Sun Prairie, Wisconsin, where I provided technical assistance, troubleshooting services, product support, and training to local dairy cooperatives, sales staff of the local dairy cooperatives, and dairy farm clients of the local dairy cooperatives.
10. From 2000 to the present, I have been Dairy Research Manager for Land O' Lakes Research Farm of Webster City, Iowa, where I am responsible for design, implementation, and summarizing dairy research trials for the farm's dairy herd that

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includes 240 cows. Additionally, as Dairy Research Manager, I assist in new feed product development and coordinate with the Feed Division of Land O' Lakes, Inc. regarding feeding trial results. Finally, as Dairy Research Manager, I am responsible for my group's annual budget of approximately \$1,100,000.00.

11. A brief resume of my educational career and my professional career from 1981 to the present is attached hereto as Exhibit A.
12. I am experienced in dairy herd feeding experiments and trials, including both *in vitro* and *in vitro* approaches, due to my education and work experience relating to dairy nutrition issues over the past 18 years.
13. The term *in vitro* describes "a biological reaction which can be performed outside the living organism in the laboratory; as, in a test tube or petri dish, on a microscope slide, etc.", whereas the term *in vivo* describes "a reaction which takes place within the living organism," according to Grant, Roger and Grant, Claire, Grant & Hackh's Chemical Dictionary, page 307 (5th edition McGraw-Hill Book Company), which is attached to this Declaration as Exhibit B.
14. *In vivo* experiments exploring the function of microorganisms in the rumen of living animals and *in vitro* experiments exploring the function of rumen microorganisms in a laboratory and isolated from the rumen of the animal are described in Hobson, P.N. The Rumen Microbial Ecosystem, pages 461-463 (1998, 1st Edition, Elsevier Science Publishers Ltd.), which is attached to this Declaration as Exhibit C. Exhibit

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C further indicates that *in vitro* results are not always reproducible under *in vivo* conditions in the rumen of a ruminant.

15. Similarly, Hobson, P.N. and Stewart C.S., The Rumen Microbial Ecosystem, pages 661-662 (1997, 2nd Edition, Chapman and Hall), which is attached to this Declaration as Exhibit D, states that *in vitro* conditions “might reproduce metabolic pathways of rumen microorganisms” that occur under *in vivo* conditions, but do not always or necessarily reproduce *in vivo* results.
16. As support for the facts provided in Paragraph 15 of this Declaration, Exhibit D states that *in vitro* experiments “could not always” quantitatively reproduce metabolic reactions of rumen microorganisms observed under *in vivo* conditions and further states: “The only ‘container’ that reproduces the rumen is the rumen.”
17. Exhibit C posits some reasons why rumen microorganism reactions under *in vitro* conditions do not always reproduce rumen microorganism reaction results observed under *in vivo* conditions. For example, Exhibit C states:

The fact that the reactions *in vitro* do not reproduce those *in vivo* does not necessarily mean that the test organism has no place in the rumen system; it may be that it’s growth conditions in the rumen have not been properly reproduced in the laboratory, or it may mean that it’s growth in the rumen is overshadowed by some unisolated organism.”
18. Indeed, as observed in Hobson, P.N., and Stewart, C.S., The Rumen Microbial Ecosystem, page 496 (1997, 2nd Edition, Chapman and Hall), which is attached to this Declaration as Exhibit E, the rumen is a complex system with many interrelated sub-systems:

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The rumen has a complex structure and the contents are heterogeneous, consisting primarily of a microbial suspension in free liquid, a solid mass of digesta, and a gas phase. Each of these entities is complex; the properties of microorganisms in suspension deep in the rumen might be different from those of microorganisms close to the rumen wall (Cheng and Costerton, 1980.) The solid mass of digesta is certainly heterogeneous, and even the gas phase is complex, consisting of a large gas space (gas-cap), smaller pockets of gas entrapped within the solid mass, and the dissolved gas. Many properties of individual parts of the microbial system of the rumen have been investigated, but often the investigations were governed by the ease of experimentation rather than the importance of the given component. This is why the complex, and relatively inaccessible, semi-solid mass of digesta has received so little attention. In addition, worthwhile investigations were hampered by a lack of simple conceptual basis for dealing with such a complex system.

19. Further factual explanation about the inconsistencies and variations observed between *in vitro* results versus *in vivo* results in relation to rumen metabolism, as mentioned in Paragraphs 14 to 17 above, is provided in Hobson, P.N. and Stewart, C.S., The Rumen Microbial Ecosystem, page 518 (1997, 2nd Edition, Chapman and Hall), attached to this Declaration as Exhibit F, which states:

The microbial system of the rumen is not a simple fermentation vat filled with randomly distributed mixtures of microorganisms. It is a highly structured and functionally compartmented system, on a par with any of the organs of the advanced multicellular organism. Significant progress in the field of rumen metabolism is contingent on appreciation of the rumen and its microbial ecosystem in this manner.

20. Continuing, Hobson, P.N., The Rumen Microbial Ecosystem, page 428 (1988, 1st Edition, Elsevier Science Publisher's Ltd.), which is attached to this Declaration as Exhibit G, explains that complex "interrelations within the system of rumen

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metabolism,” such as “microbial growth yield” issues and “digesta kinetics” issues make “the study of rumen optimization a very laborious task” and suggests that this complexity of interrelations within the rumen metabolism system is the primary reason for variability and sometimes contradictory results between *in vitro* and *in vivo* systems regarding rumen metabolism:

The complexity of the rumen and the ruminant systems to be manipulated is the main reason for variability and contradiction in experimental results.

21. As one example of the variability that may be expected between *in vitro* and *in vivo* experimental results relating to rumen metabolism, Hobson, P.N. and Stewart C.S., The Rumen Microbial Ecosystem, pages 586-587 (1997, 2nd Edition, Chapman and Hall), which is attached to this Declaration as Exhibit H, states that fiber digestibility upon isoacid supplementation showed a positive response under *in vitro* conditions, but showed only a minimal or insignificant response under *in vivo* conditions during a rumen metabolism study.
22. Next, Hobson, P.N., The Rumen Microbial Ecosystem, pages 140-141 (1988, 1st Edition, Elsevier Science Publishers Ltd.), which is attached to this Declaration as Exhibit-I, observed that added haem created a zoosporogenesis under *in vivo* rumen conditions, “but this could not be satisfactorily repeated *in vitro* with pure cultures, suggesting that the control of zoosporogenesis and zoospore really may be more complex than suggested by the work *in vivo*.” (Emphasis added.)
23. Finally, even where rumen system complexity is not a factor, Santoro, Luiz G., Grant, George, and Pusztai, A. Arpad, Differences in the Degradation *in Vivo* and *in Vitro*

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of Phaseolin, the Major Storage Protein of *Phaseolus Vulgaris* Seeds, pages 612-613, Biological Society Transactions - 625th Meeting (London 1998), which is attached to this Declaration as Exhibit J, demonstrates that large quantitative variations between *in vivo* results and *in vitro* results may nevertheless occur. Specifically, in the study that was the subject of Exhibit H, digestive degradation of phaseolin (glycoprotein II, G-II) under *in vivo* conditions was about 74%, while the extent of digestive degradation of phaseolin under *in vitro* conditions in hamsters was only about 2%, which is vastly different from the results obtained under *in vivo* conditions.

24. Thus, the collective teaching of the factual observations and statements in Paragraphs 14 to 22 above is that *in vitro* simulations of *in vivo* rumen metabolism, at least from a quantitative perspective, and possibly from other perspectives, are not necessarily a reliable predictor of *in vivo* rumen metabolism results, but are instead often speculative. Furthermore, the factual observations and statements of Paragraphs 14 to 22 above demonstrates that, for the same substrate, widely different quantitative results may be observed when comparing *in vitro* rumen metabolism experimental results to actual *in vivo* rumen metabolism results.
25. I am familiar with the disclosure of U.S. Patent No. 4,127,676 to Merensalmi (the "Merensalmi Patent"), which is attached to this Declaration as Exhibit K.
26. In Example 1, the Merensalmi Patent presents *in vitro* testing results regarding sugar alcohol preservation "in rumen fluid." (Column 2, lines 59-63, of the Merensalmi Patent of Exhibit K).

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27. The Merensalmi Patent does not provide any details whatsoever regarding the source, composition, handling, or preservation of the rumen fluid used in the *in vitro* testing referenced in paragraph 26 above, does not provide any details whatsoever about the experimental protocol or procedure for the Example 1 *in vitro* testing of sugar alcohol preservation in “rumen fluid,” and does not even provide any details about the ratio in Example 1 of the amount of rumen fluid versus the overall amount of sugar alcohol or versus the amount of individual sugar alcohols.

28. Nonetheless, despite providing no evidence about the *in vitro* testing protocol or procedure, despite providing no evidence about the source, composition, handling, or preservation of the rumen fluid, and despite providing no information about the ratio of “rumen fluid” to sugar alcohol(s), the Merensalmi Patent nevertheless alleges that the *in vitro* testing results of Example 1 conclusively establish that similar sugar alcohol degradation rates to those presented in Example 1 *would* occur under *in vivo* conditions in the rumen of a living ruminant:

The present invention, however, is based upon the prior unknown fact that sugar alcohols remain intact also under the conditions in the rumen sufficiently long without breaking down, which appears from the [in vitro] test results presented in Example 1.”

(Column 2, lines 53-57, of the Merensalmi Patent of Exhibit K; emphasis added).

29. Furthermore, the Merensalmi patent provides no evidence or reasoning whatsoever in support of the statement recited in Paragraph 28 above alleging that the *in vitro* testing results of Example 1 conclusively establish that similar sugar alcohol degradation rates to those presented in Example 1 *would* occur under *in vivo* conditions in the rumen of a living ruminant.

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30. Based upon the collective teaching of the factual observations and statements in Paragraphs 14 to 22 above about *in vitro* simulations of rumen metabolism, at least from a quantitative perspective, not being a reliable predictor of *in vivo* rumen metabolism results, and based upon my factual observations in Paragraph 28 and 29 above, the allegation recited from the Merensalmi patent in Paragraph 28 above is an unsupported and inconclusive allegation that does not actually disclose, or support a conclusion that similar sugar alcohol degradation rates to those presented in Example 1 *would* occur under *in vivo* conditions in the rumen of a living ruminant. Instead, the factual observations and statements of Paragraphs 14 to 22 above demonstrate that, for the same substrate, such as the individual sugar alcohols of Merensalmi Example 1, widely different quantitative results may be observed when comparing *in vitro* rumen metabolism experimental results to actual *in vivo* rumen metabolism results.
31. Building upon my factual observations of Paragraph 30, the Merensalmi Patent approach of equating the *in vitro* sugar alcohol degradation results of Merensalmi Example 1 to real life *in vivo* sugar alcohol degradation results that allegedly *would* be obtained under *in vivo* conditions using the complex rumen function of a live ruminant is mere speculation, since “The only ‘container’ that reproduces the rumen is the rumen.” (See Exhibit D).
32. Indeed, based upon my factual observations and factual conclusions referenced in Paragraphs 27 and 29 to 31 above, the Merensalmi tactic of equating the *in vitro* results from Merensalmi Example 1 to results that *would allegedly* be obtained under *in vivo* conditions is speculative at best and does not in fact disclose, establish, or

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prove sugar alcohol degradation rates that would be experienced under *in vivo* conditions.

33. Based on my factual observations of paragraph 32 above, it follows that the tabular *in vitro* results that extend from column 2, line 65, through column 3, line 5, of the Merensalmi Patent pertain purely to the results of the Merensalmi *in vitro* testing under unspecified conditions and unspecified "rumen fluid" to sugar alcohol ratios using an unspecified testing protocol and do not establish, prove, or disclose anything about sugar alcohol degradation in the rumen of a living ruminant under *in vivo* conditions.
34. Example 2, which extends from line 12 to line 38 in column 3 of the Merensalmi Patent, provides additional test results for a sugar alcohol mixture, as opposed to the test results provided in Example 1 of the Merensalmi Patent for individual sugar alcohols.
35. Merensalmi Example 2, like Merensalmi Example 1, refers to testing "in the rumen fluid," as opposed to *in vivo* testing in the rumen of a live ruminant, and therefore, like the test results of Merensalmi Example 1, merely amounts to *in vitro* experimentation regarding degradation characteristics of a sugar alcohol mixture in "the rumen fluid."
36. The Merensalmi patent does not provide any details whatsoever regarding the source, composition, handling, or preservation of the rumen fluid used in the *in vitro* testing referenced in paragraphs 34 and 35 above, does not provide any details whatsoever

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about the experimental protocol or procedure for the Example 2 *in vitro* testing of the sugar alcohol mixture preservation in "rumen fluid," and does not even provide any details about the ratio in Example 2 of the amount of rumen fluid versus the overall amount of sugar alcohol or versus the amount of individual sugar alcohols

37. Furthermore, the Merensalmi patent provides no evidence or reasoning whatsoever that would support an allegation that the *in vitro* testing results of Merensalmi Example 2 conclusively establish that similar sugar alcohol degradation rates to those presented in Example 2 *would* occur under *in vivo* conditions in the rumen of a living ruminant.

38. Despite the complete lack of protocol, procedure, reasoning and evidence, Merensalmi, as with Merensalmi Example 1, alleges the results of the Merensalmi Example 2 *in vitro* testing equate to results that *would* be obtained if the sugar alcohol mixture were instead subjected to *in vivo* testing in the rumen of a living ruminant:

As the flow of the fluid in the rumen is only a few hours, the sugar alcohol reaches the latter stomach compartments before any essential degradation in the rumen can occur.

(Column 3, lines 30-33, of the Merensalmi Patent of Exhibit K).

39. Nonetheless, despite this Merensalmi allegation that is recited in Paragraph 38 above, the results of the *in vitro* testing of Merensalmi Example 2, for reasons analogous to those provided in Paragraphs 30 to 32 above with regard to Merensalmi Example 1, do not establish, prove, or disclose sugar alcohol degradation rates under *in vivo* conditions in the rumen of a living ruminant and do not establish, prove, or disclose sugar alcohol degradation rates that *would* necessarily be expected upon replacing the

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in vitro procedure of Example 2 with a true *in vivo* procedure in the rumen of a living ruminant.

40. Instead, for reasons analogous to those provided in Paragraphs 30 to 32 above with regard to Merensalmi Example 1, the attempt of Merensalmi Example 2 to equate the *in vitro* sugar alcohol degradation rate results to *in vivo* results is purely speculative and without any evidence or basis in fact.

41. Finally, the last sentence in Example 4 of the Merensalmi Patent states:

The major part of the sugar alcohol mixture however passes through the rumen without breaking down.

(Column 4, lines 45-47, of the Merensalmi Patent of Exhibit K).

42. However, the only basis for this statement of Merensalmi Example 4 that is recited in Paragraph 38 above, are the erroneous and unsupported allegations of the Merensalmi patent regarding Examples 1 and 2 that are mentioned in Paragraphs 28 and 38 above.

43. For reasons analogous to those provided in Paragraphs 30 to 33 and 39 to 40 above, there is no evidence or factual basis in support of the Merensalmi Example 4 statement that is recited in Paragraph 41 above; consequently, for reasons analogous to those provided in Paragraphs 30 to 33 and 39 to 40 above, the statement recited in Paragraph 41 above regarding Merensalmi Example 4, like the prior Merensalmi statements equating the *in vitro* results of Examples 1 and 2 to *in vivo* results, is purely speculative and without any basis in fact.

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44. Ultimately, considering the factual statements and factual observations provided in Paragraphs 26 to 43, the Merensalmi patent only provides details about *in vitro* sugar alcohol degradation testing under unspecified conditions and an unspecified testing protocol and does not establish, prove, or disclose anything about sugar alcohol degradation in the rumen of a living ruminant under *in vivo* conditions.
45. I am also familiar with the disclosure of British Patent Application No. 2,159,690A of Huchette et al. (the “Huchette application”), which is attached to this Declaration as Exhibit L.
46. The Huchette application, at page 1, line 41, refers to French Patent No. 2,344,233 (the “French patent”).
47. A copy of an INPADOC Record for French Patent No. 2,344,233 obtained from an on-line search of the Delphion, Inc. patent document database is attached to this Declaration as Exhibit M.
48. According to the INPADOC Record for French Patent No. 2,344,233 that is referenced in Paragraph 47 above, French Patent No. 2,344,233 claims priority from Finnish Patent Application No. 760,746 that was filed on March 19, 1976.
49. A copy of examined Finnish Patent Application No. FI0053394B (the “Finnish application”) that is based on Finnish Patent Application No. 760,746 (referred to in Paragraph 48 above) is attached to this Declaration as Exhibit N.

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50. The Merensalmi Patent of Exhibit K, like French Patent No. 2,344,233, also claims priority from Finish Patent Application No. 760,746.
51. An INPADOC Record obtained from an on-line search of the Delphion, Inc. patent document database for examined Finnish Patent Application No. FI0053394B that is based on Finish Patent Application No. 760,746 is attached to this Declaration as Exhibit O.
52. The INPADOC Record of Exhibit O for examined Finnish Patent Application No. FI0053394B states that Finnish Patent Application No. 760,746 (referred to in Paragraphs 49 and 51 above), French Patent No. 2,344,233 (referred to above in Paragraphs 46-47), and the Merensalmi Patent of Exhibit K are all members of the same family. Correspondingly, the Merensalmi patent is believed to be an English language equivalent of both Finnish Patent Application No. 760,746 and French Patent No. 2,344,233, and the Merensalmi patent, Finnish Patent Application No. 760,746, and French Patent No. 2,344,233 are therefore believed to each contain the same disclosure or substantially the same disclosure.
53. At page 1, lines 53-64, the Huchette patent of Exhibit L addresses the French patent:
According to the explanation given [in French Patent No. 2,344,233], the effect on the milk production obtained by the addition of xylitol mother liquors to the forage is due to the fact that polyalcohols of a glucidic character have an excellent resistance to degradation in the rumen and that they would thus be capable of reaching the intestine before a considerable degradation occurs. The in vitro study of the resistance to degradation of the different polyols concerned has led to the observation that pentitols (xylitol and arabitols) offer by far the best resistance, whereas sorbitol is degraded much more rapidly.

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54. Later, at page 1, lines 70-79, the Huchette patent of Exhibit L recites:

A more recent work published in "J. Sc. Food. Agr." 1984, vol. 35, p. 21-28, relates to trials on sheep and shows also that sorbitol and mannitol disappear rapidly, especially by incubation with adapted microorganisms, and cannot be detected in the digestive contents of the duodenum, thus confirming that the effects observed from the point of view of the increase in the production of milk by administration of xylitol mother liquors to milking cows, are due exclusively to pentitols like xylitol and arabitol.

55. Finally, at page 1, lines 80-87, the Huchette patent of Exhibit L recites:

Taking into consideration the reality of this very rapid degradation of sorbitol in the rumen of ruminants, of which fact the immediate consequence is that this hexitol does not reach the duodenum, the man skilled in the art would wave aside any possibility of action on the part of the sorbitol on phenomena accompanying digestion and assimilation of foodstuffs in the case of growing cattle.

(Emphasis added).

56. The passages recited from the Huchette patent in Paragraphs 53-55 above collectively state that hexitols, such as sorbitol, degrade very rapidly in the rumen of ruminants, especially as compared to "pentitols like xylitol and arabitol," with the result that little, if any, hexitol, such as sorbitol, passes from the rumen into the duodenum upon introduction into the rumen.

57. Next, at page 2, lines 57-95, the Huchette patent of Exhibit L recites an *in vitro* example as Example 1:

EXAMPLE 1

By this example, it is shown that sorbitol is very rapidly degraded by the microorganisms of the rumen and hence cannot reach the duodenum.

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It relates to an in vitro test, carried out according to the technique developed by I.N.R.A. of Theix and in which various amounts of sorbitol are placed to incubate for six hours at 39°C in a medium not limiting in ammoniacal nitrogen and in the presence of a large amount of contents and of juice of the rumen. Samplings of the juice and of the contents of the rumen are made before feeding on a heifer provided with a fistula of the rumen and receiving a constant foodstuff regimen free from sorbitol.

The composition of the above-said medium is as follows:

-- 400 ml of artificial saliva whose composition is as follows:

58.	bicarbonate	9.24 g
59.	disodium phosphate	7.12 g
60.	K chloride	0.45 g
61.	Ca chloride	0.055 g
62.	Mg chloride	0.047 g
63.	distilled water q.s.p.	1 liter,

--200 ml of rumen juice,
--200 g of rumen contents,
--15 g of potato pulp and
--0.250 g of urea.

In three Erlenmeyer flasks, each containing a liter of this medium, are added respectively 0.40, 0.80 and 1.60 g of sorbitol.

The residual sorbitol is measured specifically in four samplings carried out respectively after 1 h, 2 h 30 minutes, 4 h and 6 h of incubation.

The results obtained are collected in Table I.

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TABLE I

	Amount (in g) of residual sorbitol in the sample taken at "t"				
	t= 0 h	t= 1 h	t= 2 h 30 mn	t= 4 h	t= 6 h
Erlenmeyer n° 1	0.40	0.25	0.02	0	0
Erlenmeyer n° 2	0.80	0.45	0.06	0	0
Erlenmeyer n° 3	1.60	1.05	0.72	0.40	0

After 2 hours and a half of incubation, the sorbitol is hence completely degraded for the doses of 0.40 and 0.80 g; with a dose of 1.60 g, it is completely degraded at the end of six hours.

58. Unlike the Merensalmi patent of Exhibit K, as mentioned above with respect to Examples 1 and 2 of the Merensalmi patent, the Huchette patent, as detailed in Paragraph 57 above, provides substantial information about the *in vitro* testing protocol, provides substantial information about the source, composition, and handling of the simulated rumen fluid, and provides substantial information about the ratio of the simulated "rumen fluid" to sugar alcohol(s) during the *in vitro* testing of Example 1 of the Huchette patent.

59. Example 1 that is recited in Paragraph 57 above from the Huchette patent demonstrates that under *in vitro* testing conditions complete degradation of hexitols, such as sorbitol, occurs in the simulated rumen fluid within a period of two and a half hours to six hours after introduction into the simulated rumen fluid, depending upon the concentration of sorbitol that is employed in the simulated rumen fluid. These *in vitro* results of Example 1 from the Huchette patent demonstrate that, upon replication under *in vivo* conditions, little, if any, hexitol, such as sorbitol, will pass

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from the rumen into the duodenum after introduction of hexitol, such as sorbitol, into the rumen of a ruminant.

60. My observations of Paragraphs 58 and 59 above, in combination with the details of Example 1 from the Huchette patent, further demonstrate the speculative, and in fact erroneous, nature of any conclusions in the Merensalmi patent (column 2, lines 53-57) about hexitols (i.e. mannitol, dulcitol, sorbitol) listed in Example 1 of the Merensalmi Patent allegedly surviving under the unspecified *in vitro* conditions of Merensalmi Example 1 and about similar sugar alcohol degradation rates to those presented in Merensalmi Example 1 *allegedly* occurring under *in vivo* conditions in the rumen of a living ruminant.
61. My observations of Paragraphs 58-59 in combination with the observations in Example 1 of the Huchette patent, when compared with the assertions from Merensalmi *in vitro* Examples 1 and 2, demonstrate wide variations in conclusions that may arise when attempting to simulate *in vivo* conditions using an *in vitro* procedure, depending upon the selected *in vitro* conditions, and further support the observation of Paragraph 24 that *in vitro* simulations of *in vivo* rumen metabolism, at least from a quantitative perspective, and possibly from other perspectives, are not necessarily a reliable predictor of *in vivo* rumen metabolism results, but are instead often speculative.

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62. Next, at page 2, line 96, through page 3, line 13, the Huchette patent of Exhibit L recites an *in vivo* example as Example 2:

EXAMPLE 2

To confirm the results presented in Example 1, the fate of the sorbitol in the rumen was studied in the Laboratory of Ruminant Digestion of the I.N.R.A. at Theix on three sheep each bearing two canulae, one to the rumen and the other to the duodenum.

40 g of sorbitol was introduced in a single dose to the rumen through the canula of the rumen before the morning feeding.

Samplings of juice and of contents of the rumen were carried out after 15 minutes, 30 minutes, 90 minutes, 2 hours, 3 hours, 4 hours, 6 hours and 8 hours.

At the same moments, samples were taken at the level of the duodenum.

The determination of the sorbitol in these samples showed:

--that the sorbitol disappeared very rapidly from the rumen, the ratio of residual sorbitol being below the detection threshold 90 minutes after administration.

--that the amount of sorbitol, which arrives in the small intestine, is very low, that is to say of the order of 2% of the amount administered, despite the size of this amount administered in a single dose.

63. Unlike the Merensalmi patent, as mentioned above with respect to Examples 1 and 2 of the Merensalmi patent, the Huchette patent, as detailed in Paragraph 62 above, actually provides, as a factual check to the *in vitro* test of Huchette Example 1, an *in vivo* example with a significant sorbitol dosage of 40 grams that demonstrates

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essentially complete degradation of hexitols, such as sorbitol, occurs in rumen fluid in the rumen itself within a period of one and a half hours after introduction of the hexitol into the rumen. These *in vivo* results of the Huchette patent confirm the observations of Paragraph 59 above about the *in vitro* results of the Huchette patent demonstrating that, upon replication under *in vivo* conditions, little, if any, hexitol, such as sorbitol, will pass from the rumen into the duodenum after introduction of hexitol, such as sorbitol, into the rumen of a ruminant.

64. The observations of Paragraph 64 above, in combination with Examples 1 and 2 from the Huchette patent, further demonstrate the speculative, and in fact erroneous, nature of any conclusions in the Merensalmi patent (column 2, lines 53-57, of the Merensalmi Patent of Exhibit K) about hexitols (i.e. mannitol, dulcitol, sorbitol) listed in Example 1 of the Merensalmi Patent surviving under appropriate *in vitro* conditions and about similar sugar alcohol degradation rates to those presented in Merensalmi Example 1 *allegedly* occurring under *in vivo* conditions in the rumen of a living ruminant.

65. The *in vitro* results of Example 1 of the Huchette patent provided in Paragraph 57 above clearly contradict the speculative conclusions posited in the Merensalmi patent about the meaning of the *in vitro* results of Examples 1 and 2 of the Merensalmi patent; furthermore, in light of Example 1 of the Huchette patent providing substantial information about the *in vitro* testing protocol, about the source, composition, and handling of the rumen fluid, and about the ratio of “rumen fluid” to sugar alcohol(s) during the *in vitro* testing of Example 1 of the Huchette patent versus the utter lack of information in the Merensalmi patent about the *in vitro*

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testing protocol; information about the source, composition, and handling of the rumen fluid; and information about the ratio of “rumen fluid” to sugar alcohol(s) during the *in vitro* testing of Examples 1 and 2 of the Merensalmi patent; the *in vitro* results of Example 1 of the Huchette patent clearly establish that the results of the *in vitro* testing and the conclusions about the *in vitro* results of Examples 1 and 2 of the Merensalmi patent are speculative, are without basis, and are in fact erroneous, at least with regard to hexitols, such as sorbitol.

66. The *in vivo* results of Example 2 of the Huchette patent provided in Paragraph 62 above, especially considering the utter lack of *in vivo* sugar alcohol degradation results in the Merensalmi patent, clearly contradict the speculative conclusions posited in the Merensalmi patent about extension of the *in vitro* results of Examples 1 and 2 of the Merensalmi patent to *in vivo* conditions, at least with regard to hexitols, such as sorbitol, and vividly demonstrate that the Merensalmi conclusions about *in vivo* applications drawn from the *in vitro* results of Examples 1 and 2 of the Merensalmi patent are speculative, are without basis, and are in fact erroneous, at least with regard to hexitols, such as sorbitol.

67. My observations of Paragraphs 64-66 in combination with the observations in *in vitro* Example 1 of the Huchette patent and *in vivo* Example 2 of the Huchette patent, when compared with the assertions from Merensalmi *in vitro* Examples 1 and 2, demonstrate wide variations in conclusions that may arise when attempting to simulate *in vivo* conditions using an *in vitro* procedure, depending upon the selected *in vitro* conditions, and further support the observation of Paragraph 24 that *in vitro* simulations of *in vivo* rumen metabolism, at least from a quantitative perspective, and

First Named Inventor: Cindie M. Luhman

Application No.: 09/239,873

Declaration of Paul A. Porter under 35 U.S.C. §132

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possibly from other perspectives, are not necessarily a reliable predictor of *in vivo* rumen metabolism results, but are instead often speculative.

68. Considering my observations of Paragraphs 64-67, the *in vivo* results from Example 2 of the Huchette patent, whether or not considering the *in vitro* results from Example 1 of the Huchette patent, ultimately demonstrate and highlight the speculative and erroneous nature of the conclusions posited in the Merensalmi patent regarding the *in vitro* results of Examples 1 and 2 of the Merensalmi patent, at least with regard to hexitols, such as sorbitol.

I declare that all statements made herein that are of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Inventor: Paul A. Porter

(Printed Name)

Inventor: 

(Signature)

Date

1/3/03

Applicant	: Cindie M. Luhman	
Serial No.	: 09/239,873	
Filed	: January 29, 1999	Group Art Unit: 1616
For	: METHOD AND COMPOSITION FOR ENHANCING MILK COMPONENT CONCENTRATIONS	Examiner: N. Levy
Docket No.	: LL11.12-0040	

EXHIBIT A

of

DECLARATION UNDER 35 U.S.C. § 132

Resume of the Declarant, Paul A. Porter

Paul A. Porter, Ph.D.
1025 190th St.
Webster City, IA 50595
515 543-4852 x222

Education:

Ph.D., Animal Science (Dairy Nutrition emphasis), Cornell University, Ithaca, NY. 1987.
M.S., Animal Science, Cornell University, Ithaca, NY. 1984.
B.A., Chemistry, Wittenberg University, Springfield, OH. 1981.

Professional Experience:

2000-present

Dairy Research Manager, Land O' Lakes Research Farm, Webster City, IA
Responsible for design, implementation and summary of research trials in the 240-cow dairy herd; present summarized information to the Feed Division and assist in new product development; manage \$700,000 annual budget.

1994-2000

Dairy Nutritionist and Field Technical Services Manager, Land O' Lakes Dairy Feed, Sun Prairie, WI
Provided technical assistance, troubleshooting, product support and training to local cooperatives, their sales staff and their dairy farm clients. Developed sales tools such as spreadsheet economic evaluations and new product information materials.

1992-1994

Dairy Sales & Consulting, Young's Livestock Nutritional Services, Canastota, NY
Provided management and production record analysis & consulting and designed customized feeding programs for dairy farm clients packaged with sales of mineral products.

1989-1992

Dairy Program Manager, Countrymark Coop, Delaware, OH
Provided technical assistance, troubleshooting, product support and training to local cooperatives, their sales staff and their dairy farm clients. Developed sales tools such as spreadsheet economic evaluations and new product information materials.

1987-1989

Assistant Professor, Department of Animal Science, Oregon State University, Corvallis
Conducted applied dairy nutrition research and taught courses in ration balancing, dairy herd management and advanced dairy herd management; acted as liaison to state dairy industry and presented papers at numerous meetings.

Memberships:

American Dairy Science Association
American Registry of Professional Animal Scientists (ARPAS)

Applicant	: Cindie M. Luhman	
Serial No.	: 09/239,873	
Filed	: January 29, 1999	Group Art Unit: 1616
For	: METHOD AND COMPOSITION FOR ENHANCING MILK COMPONENT CONCENTRATIONS	Examiner: N. Levy
Docket No.	: LL11.12-0040	

EXHIBIT B

of

DECLARATION UNDER 35 U.S.C. § 132

Grant, Roger and Grant, Claire, Grant & Hackh's Chemical Dictionary,
page 307 (5th edition McGraw-Hill Book Company)

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intramuscular Inside muscular tissue; as, of an injection.
intranuclear (1) Within an atomic nucleus. (2) Within a molecular ring system. *i. tautomerism* The shifting of a double bond within one or more rings.

Intraval sodium Trademark for thiopental sodium.

intravenous Within veins, e.g., an injection.

intravital (1) Within the living organism. (2) Within a lifetime.

introduction Causing the entry of a different type of atom into an organic molecule, e.g., chlorination.

introfaction A change in the fluidity and specific wetting properties of an impregnating material, due to an introfier.

introfier Impregnation accelerator. A substance that speeds up the penetrating power of fluids.

intrusion Forcing a material into the cavities or pores of a substance.

intumescence (1) Swelling up, especially of certain crystals on heating. (2) Popping, puffing. The violent escape of moisture on heating.

inula camphor Helenin.

inulenin (C₆H₁₀O₅)_n·2H₂O. A carbohydrate associated with inulin. Colorless needles, soluble in water.

inulic acid Alantonic acid.

inulin C₆H₁₁O₅(C₆H₁₀O₅)_nOH. Alantoin, alant starch, dahlin, sinistrin. A polysaccharide from the rhizome of *Inula helenium* or *Dahlia variabilis*. White powder, m.160 (decomp.), soluble in hot water. Used to measure glomerular filtration rate of kidneys.

inulinase An enzyme that endohydrolyzes 2,1-β-D-fructosidic linkages in inulin.

in vacuo In a vacuum, q.v.

Invar Trademark for the ferronickel: Ni 36, steel 64% (carbon content 0.2%), d.8.0, m.1500. It has a low coefficient of heat expansion; used for precision instruments.

inversion (1) The turning of a levo to a dextro compound, or vice versa. (2) The change of an isomeric compound to its opposite, as a *cis* to a *trans* compound. (3) The hydrolysis of an optically active disaccharide to 2 optically active monosaccharides; e.g., the hydrolysis of cane sugar to glucose and fructose by dilute acids, alkalies, or enzymes, resulting in a change in the direction and degree of rotation of polarized light. Cf. *Walden inversion*, *Clerget inversion*. (4) In an emulsion of 2 immiscible liquids, the interchange of the internal and external phases. **dipole** ~ Symmetrization. The reversal of the normal activity of functional groups in organic chemistry.

i. point The temperature at which *i.* takes place.

invertase β-D-Fructofuranosidase*, saccharase, invertin. An enzyme of the pancreatic juice and of yeast, which hydrolyzes terminal, nonreducing β-D-fructofuranoside residues in β-D-fructofuranosides; converts cane sugar into invert sugar.

invertin Invertase.

invert soap A cationic, surface-active detergent, so called because it ionizes oppositely to soap; e.g., quaternary ammonium or sulfonium compounds.

invert sugar Approximately 50% glucose and 50% fructose, obtained by the acid hydrolysis of cane sugar. It is slightly levorotatory, fermentable; it reduces Fehling's solution and is used in brewing. *i. s. solution* A partially inverted solution of sucrose containing at least 62% solids, 3–50% *i. s.*, and equal weights of fructose and glucose.

in vitro Describing a biological reaction which can be performed outside the living organism in the laboratory; as, in a test tube or petri dish, on a microscope slide, etc. Cf. *in vivo*.

i. v. fertilization I.V.F. Fertilization in the laboratory of a

(human) ovum, removed from an ovary, by sperm. (Used in conception of "test tube babies.") See *embryo replacement*.

in vivo Describing a reaction which takes place within the living organism. Cf. *in vitro*.

inyolite 2CaO·3B₂O₃·13H₂O. A native borate (S. California).

iod- See *iodo-*.

iodal Cl₃·CHO = 421.7. A liquid resembling chloral.

iodalbumin A red compound of blood albumin and iodine, of molasseslike odor.

iodaniline Iodoaniline*.

iodate A salt of iodic acid, containing the radical IO₃⁻.

iodoerin C₂₀H₅O₅I₄ = 835.9. Erythrosin, tetraiodofluorescein. A red indicator powder, soluble in alcohol (alkalies—rose-red, acids—yellow). *i. solution* A 0.0002% solution of iodoerin in ether. This is added to dilute alkali and titrated until the rose tint passes from the ether into the aqueous solution.

iodi- See *iodo-*.

iodic i. acid HIO₃ = 175.9. Metaiodic acid. Colorless rhombs, m.110, soluble in water. Used as an oxidizing agent; as a reagent for alkaloids, biliary pigments, naphthol, thiocyanates, and guaiacol; in organic synthesis, and for volumetric solutions *per* ~ See *periodic acid*. *i. anhydride* Iodine pentoxide*.

iodide M_I. A binary compound of iodine with a metal. *i. ion** The I⁻ ion.

iodimetry Iodometry.

iodinated (¹³¹I) serum A sterile solution of human serum albumin, treated with ¹³¹I and freed from iodide; used to diagnose lung conditions; as, small tumors or emboli, and to estimate blood volume.

iodine I = 126.9045. Id* (if I* is inconvenient). Iodum. A nonmetallic element, at. no. 53, of the halogen group. Rhombic, bluish-black, lustrous plates or scales, d.4.948, m.114, b.184, slightly soluble in water, soluble in alcohol or iodide solutions. Discovered by Courtois (1811) and named after its purple vapors (Greek: *iodēs*, the "violet" and *ion*, "similar"). Obtained from the mother liquor of Chile saltpeter and seaweed ash, and widespread in nature. Valency: usually 1 (iodides*), or 3 (iodonium*), or 5 (iodates*). Used as a reagent in volumetric analysis; in organic synthesis; in the manufacture of iodides, iodates, and iodine preparations; and as an antiseptic and caustic. Used medically (¹²⁵I and ¹³¹I) as sodium iodide and iodinated albumin. I. is also an essential trace element, present in thyroid hormones; deficiency in diet leads to goiter and hypothyroidism. Recommended daily intake 150 μg. *eka* ~ Early name for astatine.

povidone ~ (C₆H₉ON)_nI. 1-Vinyl-2-pyrrolidinone polymer with iodine. Betadine. An antiseptic (USP, EP, BP). **solution of** ~ (1) Lugol solution. (2) Colorless Lugol solution; decolorized with sodium thiosulfate. (3) *Iodine water*, q.v. **tincture of** ~ An alcoholic 7% iodine solution in 5% potassium iodide solution; an antiseptic (USP).

i. acetate IC₂H₃O₂ = 185.9. A solid prepared from chlorine dioxide and *i.* in glacial acetic acid. *i. bromides* IBr, *i. monobromide*; IBBr₃, *i. tribromide*; IBBr₅, *i. pentabromide*. *i. chlorides* ICl, *i. monochloride*; ICl₃, *i. trichloride*. *i. cyanide** ICN = 152.9. Cyanogen *i.* Colorless crystals, m.146, soluble in water. *i. cycle* See Fig. 15. *i. dioxide** IO₂ = 158.9 or I₂O₄ = 317.8. Yellow powder, decomp. into its elements at 130. *i. disulfide* Sulfur iodide. *i. green* A phenolphthalein dye pH indicator, changing at 1.0 from yellow (acid) to blue-green (alkaline); also stains liquefied xylem in plant tissues. *i. fluoride* See *iodine pentafluoride*. *i. monobromide** IBR = 206.8. Purple crystals, m.36, soluble in water (decomp.). Used

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Roger L. Grant, whose father prepared the 4th edition.

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Docket No.	: LL11.12-0040	

EXHIBIT C

of

DECLARATION UNDER 35 U.S.C. § 132

Hobson, P.N., The Rumen Microbial Ecosystem,
pages 461-463 (1988, 1st Edition, Elsevier Science Publishers Ltd.)

THE RUMEN MICROBIAL ECOSYSTEM

Edited by

P. N. HOBSON

*Honorary Research Fellow, Biochemistry Department,
Marischal College, Aberdeen University,
Aberdeen, UK*

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Rowett Research Institute, Aberdeen, UK*



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Applicant	: Cindie M. Luhman	
Serial No.	: 09/239,873	
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Docket No.	: LL11.12-0040	

EXHIBIT D

of

DECLARATION UNDER 35 U.S.C. § 132

Hobson, P.N. and Stewart C.S., The Rumen Microbial Ecosystem,
pages 661-662 (1997, 2nd Edition, Chapman and Hall)

The Rumen Microbial 'Ecosystem

Edited by

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*Department of Molecular and Cell Biology
University of Aberdeen*

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C.S. STEWART

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Aberdeen*

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Dedicated to B.

Applicant	: Cindie M. Luhman	
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Hobson, P.N. and Stewart, C.S., The Rumen Microbial Ecosystem,
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THE RUMEN MICROBIAL ECOSYSTEM

Edited by

P. N. HOBSON

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*Formerly Head, Microbial Chemistry Department,
Rowett Research Institute, Aberdeen, UK*



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Applicant	: Cindie M. Luhman	
Serial No.	: 09/239,873	
Filed	: January 29, 1999	Group Art Unit: 1616
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EXHIBIT J

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Santoro, Luiz G., Grant, George, and Pusztai, A. Arpad, Differences in the Degradation In Vivo and In Vitro of Phaseolin, the Major Storage Protein of *Phaseolus Vulgaris* Seeds, pages 612-613, Biological Society Transactions - 625th meeting, (London 1998)

phonic analogue is not recognized. These collected findings are consistent with recent crystallographic and chemical studies on the active site of the enzyme (Sandmeier & Christen, 1982; Arnone *et al.*, 1984; Kirsch *et al.*, 1984): the substrate specificity is largely determined by the interactions of α - and ω -carboxylate groups with guanidinium groups of Arg-386 and Arg-292, respectively. The binding of the two carboxylate groups induces a conformational change of the protein, and the ϵ -amino group of Lys-258 which binds the coenzyme can act as C_{α} -H proton acceptor. It may be assumed that the replacement of one of the carboxylate groups induces restrictions in such conformational changes, leading to a decrease in reactivity of the effector. Distinctions in cytosolic and mitochondrial isozymes with respect to the distances between the two arginyl residues and the lysyl 258 Schiff's base, and/or the degree of freedom of the rotation of these active site components (Iriarte *et al.*, 1984) could explain the different data observed for Asp- β -P and Glu- γ -P with the two isozymes.

The present results relative to the aminomalonate analogue and Asp- β -P, which are both substrates and inhibitors, show the importance of an α -carboxylic group in the binding of an effector with the ASAT active site.

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Differences in the degradation *in vivo* and *in vitro* of phaseolin, the major storage protein of *Phaseolus vulgaris* seeds

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Storage proteins of legume seeds are considered to be poorly digestible in their native form (Liener & Thompson, 1980). The partial resistance of native phaseolin (glycoprotein II, G-II), the main storage protein of *Phaseolus vulgaris*, to degradation by pepsin, trypsin and chymotrypsin, *in vitro*, alone or in a sequential combination (Liener & Thompson, 1980; Deshpande & Nielsen, 1987) has been demonstrated. Limited proteolysis by trypsin or chymotrypsin reduced the size of the native protein from 140 kDa to 120 kDa (Liener & Thompson, 1980). The subunits of G-II were cleaved near the middle of the polypeptide chains and the products in SDS had M_r values in the range of 22-30 kDa. According to Vaintraub *et al.* (1976) and R. Begbie (unpublished work), the hydrolysis *in vitro* of undenatured G-II by pepsin resulted in only about 2% of the total peptide bonds being broken. On the other hand, G-II appeared to be more extensively degraded *in vitro*, if instead of pure gut endopeptidases, stomach and small intestinal luminal and tissue extracts were used as sources of proteolytic enzymes (Sgarbieri *et al.*, 1982).

As there is much less information available about the degradation *in vivo* of pure G-II, in the present work, the true digestibility *in vivo* of a highly purified G-II preparation was studied in rats.

G-II was isolated from seeds of *P. vulgaris* as before (Pusztai & Watt, 1970). The amount of lectin contamination was reduced to less than 0.3% by affinity chroma-

tography on Sepharose 4B-fetuin. It was thought unlikely that the results would be influenced unduly by the presence of such a small amount of toxic component.

Rats fasted for 16 h were given an intragastric dose of 300 mg of G-II (45 mg N) and then fed a protein-free diet, *ad lib*, for 3 days. Faeces were collected daily and N estimated. Faeces were also extracted with 0.025 M-glycine/Tris buffer, pH 8.6 (3-5 mg of faeces/ml of buffer) and the concentration of G-II-related material was estimated by rocket immunoelectrophoresis. This was based upon previous observation (R. Begbie, unpublished work) that the fragment derived from the partial digestion of G-II retained full reactivity with anti-G-II antibodies.

It was found that (Fig. 1a) the faeces contained more than 50 mg of total N although only 45 mg of G-II N had been introduced into the stomach. The faecal N comprised approx. 12 mg of G-II N. The remainder, about 38 mg, was unrelated to G-II, indicating that 74% of the G-II was degraded during passage through gastrointestinal tract. The bulk (8 mg) of the partially digested G-II N appeared in the faeces in the first 24 h, while the output of N unrelated to G-II peaked on the second day. It appears that by an unknown mechanism, the native G-II and/or its fragments may have stimulated an increased secretion of endogenous metabolic N.

Since proteolysis by bacteria in the large intestine may have made appreciable contributions to degradation of G-II without nutritionally benefiting the rat, in another series of experiments rats were given an intragastric dose of 150 mg of G-II (22.5 mg N) and killed after 1 h. Both stomach and small intestine were removed and their contents washed out. The tissues were then homogenized in phosphate buffer containing aprotinin (5 mg tissue/ml of buffer) and centrifuged. After correction for the control (rats given an intragastric

Abbreviation used: G-II, glycoprotein II.

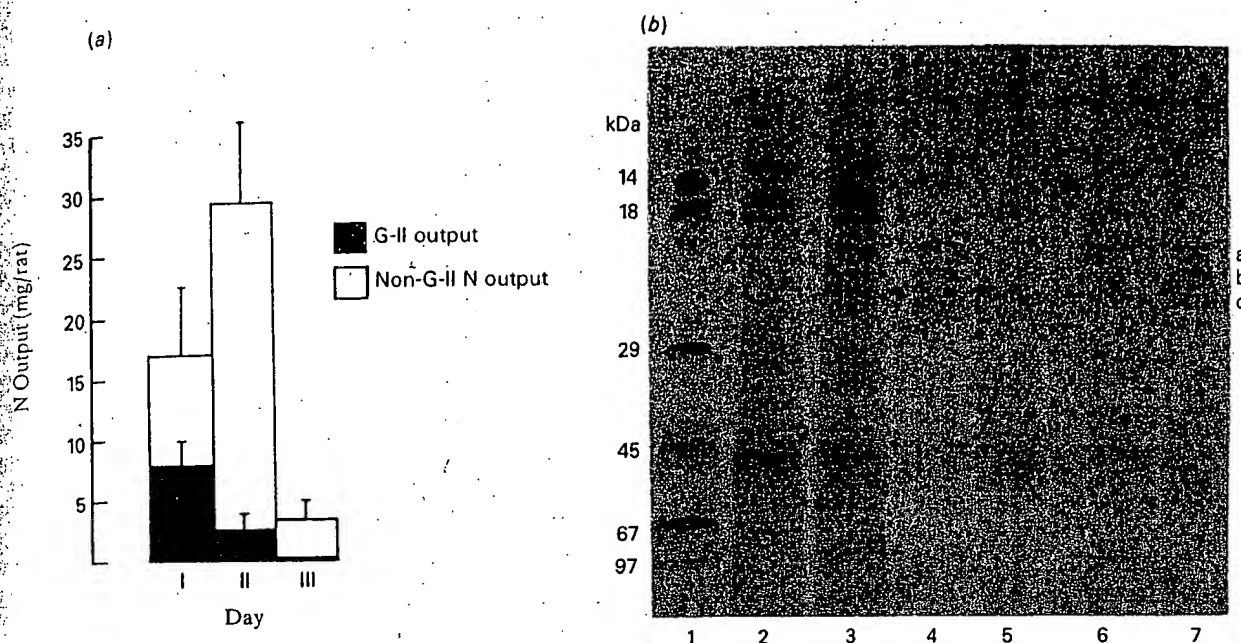


Fig. 1. (a) Total N (corrected for normal metabolic faecal N) and G-II N output over 3 days in the faeces of rats given 300 mg of G-II (45 mg N) and (b) SDS/PAGE of G-II related polypeptides.

Lane 1, standards; lane 2, extract from the small intestine tissue of control rats; lane 3, extract from the small intestine tissue of rats given an intragastric dose of G-II; lane 4, G-II core-polypeptides recovered in luminal contents of rats given G-II; lane 5, native G-II; lanes 6 and 7, G-II core-polypeptides from faeces; (a) ≈ 22 kDa, (b) ≈ 23 kDa, (c) ≈ 25 kDa.

dose of saline), the total amount of N found in the luminal contents and tissue homogenates was at least as much (23.4 ± 0.8 mg) as in the G-II input. On the other hand, the amount of G-II-related N recovered from the small intestine was only 9.6 ± 0.8 mg, indicating that about 57% of the G-II was degraded and absorbed within 1 h. Moreover, a large proportion (about 60%) of the surviving G-II-related protein was found to be strongly associated with small intestinal tissue (Fig. 1b) and was released only on homogenization.

In conclusion, at least 57% of G-II was degraded in the small intestine within 1 h. During the remaining time (≈ 1 h) in the small intestine and then in the large intestine, G-II was further degraded. The core-polypeptide fragments of 22–30 kDa surviving in the small intestine showed a similar subunit pattern by SDS/polyacrylamide gel electrophoresis (PAGE) to that observed on degradation *in vitro* (Deshpande & Nielsen, 1987). As the extent of degradation of G-II *in vivo* (74%) exceeds that obtained *in vitro* by pure endopeptidases (2%), the results strongly suggest the involvement of additional proteinases in the breakdown of G-II in the gut. The strong attachment of G-II to the intestinal tissue and extended exposure time to proteolytic enzymes may further aid this digestive process. However, an appreciable part (about 26%) of the dietary G-II escapes digestion in the whole alimentary tract. The reasons for this may be related

to the previously observed microheterogeneity of G-II (Pusztai & Stewart, 1980) or partial protection from proteolysis by an unknown mechanism.

Finally the large difference between apparent and true digestibility values indicates that G-II and/or its fragments are stimulants of secretion of endogenous N (mucus, etc.) in the small intestine.

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EXHIBIT K

of

DECLARATION UNDER 35 U.S.C. § 132

U.S. Patent No. 4,127,676 to Merensalmi

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EXHIBIT L

of

DECLARATION UNDER 35 U.S.C. § 132

British Patent Application No. 2,159,690A of Huchette et al.

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(54) **Method and agent for the optimisation of the assimilation of the feed ration by fattening ruminants**

(57) A method of optimisation of the assimilation of the feed ration in fattening ruminants, comprising possibly a maintenance period, consists in making the ruminant ingest, at the same time as the normal food ration, an effective amount of sorbitol. Foodstuffs for growing cattle can comprise an effective amount of sorbitol for example between 0.1 to 2.0% by weight.

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SPECIFICATION

Method and agent for the optimisation of the assimilation of the food ration by growing cattle

- 5 The invention relates to a method and to an agent for the optimisation of the assimilation of the food ration by growing cattle, that is to say by ruminants intended for meat production.
- 10 The invention also relates to the optimisation of the assimilation of the food ration of ruminants intended for meat production when the said ruminants are in a maintenance period, particularly the winter season.
- The invention also relates, as new industrial products, to compositions and foodstuff forms intended for said animals and incorporating this agent.
- 15 The optimisation of the assimilation of the food ration — that is to say the obtaining of an increase in weight as high and rapid as possible for a given ration — is wished for by reason of its effects from the economic point of view whatever the type of bred cattle concerned.
- It has a particular importance in the case of ruminants — particularly oxen, bull-calves, cows, 25 heifers — intended for fattening, that is to say the production of meat, by reason of the well known fact that a part only of the food is used by these animals for their growth, this by reason of the nature of foodstuffs currently distributed, which are only partly digested, as well as by reason of their particular anatomy essentially adapted to a herbivorous diet.
- It has already been proposed, to overcome this drawback, to protect, for example by tanning or by encapsulation, at least certain of the constituent 35 elements of the food ration to avoid them being too considerably degraded in the rumen and so that they can reach the duodenum.
- It has also been proposed, in a field different from that of the fattening of ruminants, namely that of 40 increasing the blood sugar content and the milk yield of ruminants (French Patent No. 2,344,233), to use xylitol mother liquors as additives for foodstuffs for milking cows; it is recalled in this respect that the alimentary diet of the milch-cow is very different from 45 that of growing cattle both in the different ratio of digestible protein to non-digestible protein, and by the addition of carbohydrates. The Xylitol mother liquors to which recourse is had, comprise with respect to the dry matter from 5% to 25% of xylitol, 50 from 20% to 35% of arabitol and 10% to 25% of mannitol, 5% to 15% of sorbitol, from 5% to 10% of dulcitol and from 5% to 10% of rhamnitol.
- According to the explanation given, the effect on the milk production obtained by the addition of xylitol 55 mother liquors to the forage is due to the fact that polyalcohols of a glucidic character have an excellent resistance to degradation in the rumen and that they would thus be capable of reaching the intestine before a considerable degradation occurs. The *in vitro* study 60 of the resistance to degradation of the different polyols concerned has led to the observation that pentitols (xylitol and arabitol) offer by far the best resistance, whereas sorbitol is degraded much more rapidly.
- 65 The fact that the xylitol mother liquors can be

considered as additive without danger and useful for the feeding of milch-cows, results also from the study, published in "NUTRITION REPORTS INTERNATIONAL", June 1981, vol. 23, n°6, p. 1077-1087.

- 70 A more recent work published in "J. Sc. Food Agr." 1984, vol. 35, p. 21-28, relates to trials on sheep and shows also that sorbitol and mannitol disappear rapidly, especially by incubation with adapted microorganisms, and cannot be detected in the digestive contents of the duodenum, thus confirming that the 75 effects observed from the point of view of the increase in the production of milk by administration of xylitol mother liquors to milking cows, are due exclusively to pentitols like xylitol and arabitol.
- 80 Taking into consideration the reality of this very rapid degradation of sorbitol in the rumen of ruminants, of which fact the immediate consequence is that this hexitol does not reach the duodenum, the man skilled in the art would wave aside any possibility 85 of action on the part of the sorbitol on phenomena accompanying digestion and assimilation of foodstuffs in the case of growing cattle.
- And it is — neither the knowledge of French patent N° 79 90 01697, which recommends the use of sorbitol, as a cholagogic agent, in the preruminant calf, which could change anything at all in this respect in the mind of the man skilled in the art since the preruminant calf has a monogastric animal physiology, 95 — nor the fact that sorbitol has already been used to complement certain vitamin solutions or certain curative preparations, the administration then being carried out either with very low doses, or with larger doses but punctually and episodically and for a limited 100 time, in the case of digestive troubles of certain animals.
- Under these conditions, the merit of Applicants is all the greater in having found that, quite surprisingly and unexpectedly, the addition of a small amount of 105 sorbitol to feedstuffs for growing cattle, that is to say for meat production, enabled the assimilation of the food ration for these animals to be optimised, in other words — to increase significantly the average daily gain in 110 weight and, simultaneously, — to improve the consumption index which is illustrated by the ratio "amount of foodstuff ingested/ amount of meat produced".
- It follows that the method according to the invention 115 of optimising assimilation of the food ration in growing cattle comprising possibly a maintenance period, is characterised by the fact that the ruminants are made to ingest or eat, at the same time as the normal foodstuff ration, an effective amount of 120 sorbitol.
- It follows also that the agent for the optimisation of the assimilation of the feedstuff ration in the growing cattle is characterised by the fact that it is essentially constituted by sorbitol of which is intended consequently the application to the abovesaid process of 125 optimisation.
- It follows finally that the foodstuff for growing cattle according to the invention is characterised by the fact that it comprises an effective amount of the abovesaid 130 agent, constituted essentially by sorbitol.

Whichever that of the various aspects of the invention defined above which is retained, the sorbitol employed can be in the form of a powder or of a solution, or pure, or in the form of a hydrogenated starch hydrolysate of which it represents the principal constituent; preferably, the sorbitol is, in the latter case, present in the proportion of at least 71% by weight, expressed on the dry matter content of the hydrolysate.

Advantageously, the amount of sorbitol employed is at least 10 g per day, the practical limit, not imperative but imposed by economic considerations, being about 200 g per day.

More precisely, the abovesaid lower limit is about 20 g and the upper limit about 129 g per day, an amount frequently selected being 80 g per day.

In an advantageous embodiment of the invention, the industrial product constituted by the foodstuff for the ruminant, comprises a proportion of about 0.1 to about 2% by weight, preferably from about 0.3% to about 1.2% of sorbitol, these percentages being expressed in dry matter to dry matter.

The use of sorbitol in the feeding of ruminants enables, as illustrated by the examples, the average daily gain in weight of the animals to be very substantially increased; what is important, is that this average daily gain is not obtained by a larger consumption of foodstuffs since the consumption index is not higher but, on the contrary, even generally lower.

The mechanism of action of the sorbitol has not yet been explained.

Tests made within the scope of the invention have shown besides, that the optimisation effect exerted by sorbitol is particularly marked with foodstuffs having contents of total nitrogenous materials which are low or average, that is to say less than 15% and, in practice, comprised between about 9% and about 13%, these percentages being expressed in $N \times 6.25$ with respect to dry matter.

A preferred embodiment of the method according to the invention consists therefore of causing the ruminant to ingest an effective amount of sorbitol at the same time as a foodstuff having a content of $N \times 6.25$ less than 15% and, preferably, comprised between 9% and 13% by weight.

The administration of the sorbitol may be done by mixing with other constituents of the food ration of the ruminant at meal times; it is possible to provide ready-for-use mixes, that is to say feed stuffs directly useable and comprising, besides the sorbitol, at least certain of, if not all the constituents of the food ration.

The invention will be still better understood by means of the examples which follow and which comprise the description of advantageous embodiments.

EXAMPLE 1

By this example, it is shown that sorbitol is very rapidly degraded by the microorganisms of the rumen and hence cannot reach the duodenum.

It relates to an in vitro test, carried out according to the technique developed by I.N.R.A. of Theix and in which various amounts of sorbitol are placed to incubate for six hours at 39°C in a medium not limiting in ammoniacal nitrogen and in the presence of a large amount of contents and of juice of the rumen. Samplings of the juice and of the contents of the rumen are made before feeding on a heifer provided with a fistula of the rumen and receiving a constant foodstuff regimen free from sorbitol.

The composition of the above-said medium is as follows:

— 400 ml of artificial saliva whose composition is as follows:

75	. bicarbonate	9.24 g
	. disodium phosphate	7.12 g
	. K chloride	0.45 g
	. Ca chloride	0.055 g
	. Mg chloride	0.047 g
80	. distilled water q.s.p.	1 liter,
	— 200 ml of rumen juice,	
	— 200 g of rumen contents,	
	— 15 g of potato pulp and	
	— 0.250 g of urea.	

In three Erlenmeyer flasks, each containing a liter of this medium, are added respectively 0.40, 0.80 and 1.60 g of sorbitol.

The residual sorbitol is measured specifically in four samplings carried out respectively after 1 h, 2 h 30 minutes, 4 h and 6 h of incubation.

The results obtained are collected in Table I.

TABLE I

	Amount (in g) of residual sorbitol in the sample taken at "t"				
	t= 0h	t= 1h	t= 2h 30 mn	t= 4h	t= 6h
Erlenmeyer n°1	0.40	0.25	0.02	0	0
Erlenmeyer n°2	0.80	0.45	0.06	0	0
Erlenmeyer n°3	1.60	1.05	0.72	0.40	0

After 2 hours and a half of incubation, the sorbitol is hence completely degraded for the doses of 0.40 and 0.80 g; with a dose of 1.60 g, it is completely degraded at the end of six hours.

EXAMPLE 2

To confirm the results presented in Example 1, the fate of the sorbitol in the rumen was studied in the Laboratory of Ruminant Digestion of the I.N.R.A. at

Theix on three sheep each bearing two canulae, one to the rumen and the other to the duodenum.

40 g of sorbitol was introduced in a single dose to the rumen through the canula of the rumen before the morning feeding.

Samplings of juice and of contents of the rumen were carried out after 15 minutes, 30 minutes, 90 minutes, 2 hours, 3 hours, 4 hours, 6 hours and 8

hours.

At the same moments, samples were taken at the level of the duodenum.

The determination of the sorbitol in these samples showed:

- that the sorbitol disappeared very rapidly from the rumen, the ratio of residual sorbitol being below the detection threshold 90 minutes after administration.
- that the amount of sorbitol, which arrives in the small intestine, is very low, that is to say of the order of 2% of the amount administered, despite the size of

this amount administered in a single dose.

EXAMPLE 3

- 15 Two in vivo tests were carried out at the Laboratory of Meat Production of I.N.R.A. at Theix on bull-calves aged eight months; two types of feed stuffs were tried, the first having corn husks as a base, the other having corn seed or grain as a base.

- 20 a) *Results obtained with the foodstuff based on corn husks*

Six feeding diets whose composition is indicated in Table II, were administered to six groups of five bull-calves.

TABLE II
COMPOSITION OF FOOD-STUFF DIETS

Constituent (Z)	Regime n° 1	Regime n° 2	Regime n° 3	Regime n° 4	Regime n° 5	Regime n° 6
Corn husks	79.1	79.7	78.2	78.8	77.3	77.9
Starch	13.1	13.2	13.0	13.1	12.8	12.9
Corn grain	5.5	5.5	5.4	5.4	5.4	5.4
Vitaminised mineral condiment	1.6	1.6	1.6	1.6	1.6	1.6
Urea	0	0	1.1	1.1	2.2	2.2
Sorbitol	0.70	0	0.70	0	0.70	0
Total nitrogenous materials (g of N x 6.25 per kg)	90.75	90.4	121.3	121.9	152.0	152.6

- 25 The results obtained with the various food stuff diets or regimens are collected in Table III.

TABLE III

Identification of the magnitude contemplated	Duration	Regimen					
		n°1	n°2	n°3	n°4	n°5	n°6
Accumulated average daily gain (in g)	56 days	1025	888	1263	1221	1096	1236
	124 days	1162	1030	1130	1200	1120	1230
	142 days	1137	1042	1193	1103	1140	1245
Gain in weight (in g)	56 days	145	1117	187	170	161	174
	124 days	153	130	157	153	150	161
Forage units ingested							

- 30 On examining the values collected in Table III, it is observed that the sorbitol improved the average daily gain and that this increase is not due to an increase in the amount of ingested foodstuff. In fact, the gain in weight per unit energy is improved when sorbitol is added.

On the other hand, for this type of foodstuff, the

- 35 sorbitol does not seem to have any effect when the content of nitrogenous material is higher than 15%.

b) Foodstuff regimen based on corn grain

The composition of the foodstuff diets is indicated in Table IV. Each regimen is administered to a group of bull-calves.

TABLE IV
COMPOSITION OF FOODSTUFFS

Constituent (Z)	Regime n° 1	Regime n° 2	Regime n° 3	Regime n° 4	Regime n° 5	Regime n° 6
Corn grain	76.9	77.4	75.9	76.5	75.2	75.0
Starch	6.7	6.8	6.8	6.7	6.5	6.6
Corn husks	12.4	12.6	12.4	12.5	12.1	12.2
Vitaminised mineral condiment	1.6	1.6	1.6	1.6	1.6	1.6
Urea	0	0	1.1	1.1	2.2	2.2
Sorbitol	0.80	0	0.80	0	0.80	0
Total nitrogenous material (g of N x 8.25 per kg)	93.4	94.1	124.0	125.0	154.6	155.3

The results obtained are collected in Table V.

TABLE V

Identification of the magnitude considered	Duration	Regimen					
		n°1	n°2	n°3	n°4	n°5	n°6
Accumulated	56 days	888	621	1107	1023	1236	1264
average daily	124 days	1000	790	1270	1130	1340	1390
gain (in g)	142 days	970	818	1232	1151	1352	1392
Gain in weight (in g)	56 days	135	88	165	144	183	178
Forage units ingested	124 days	132	102	168	146	177	181

It is here again observed that addition of sorbitol considerably improved the average daily gain and foodstuff effectiveness for animals receiving foodstuffs whose content is N x 6.25 is about 9% to 12.5%.

CLAIMS

1. A method for the optimisation of the assimilation of the feed ration in growing cattle comprising possibly a maintenance period, characterised by the fact that the ruminant is made to ingest, at the same time as the normal foodstuff ration, an effective amount of sorbitol.
2. A method as claimed in claim 1 characterised by the fact that the amount of sorbitol employed is at least 10 g per day.
3. A method as claimed in claim 1 or claim 2 characterised in that the upper limit of the amount of sorbitol employed is about 200 g per day.
4. A method as claimed in any of claims 1 to 3 characterised in that the amount of sorbitol employed is from about 20 to about 120 g per day.
5. A method as claimed in claim 4 in which the amount of sorbitol is about 80 g per day.
6. A method as claimed in claim 1 substantially as herein described.
7. Optimisation agent for the assimilation of the feed ration in growing cattle, characterised by the fact that it is essentially constituted by sorbitol.
8. Use of sorbitol as an optimisation agent for the assimilation of the feed ration in growing cattle.
9. Foodstuff for growing cattle characterised by the fact that it comprises an effective amount of the agent according to claim 7.
10. Foodstuff for growing cattle, characterised in that it contains an amount of sorbitol of between about 0.1 and about 2% by weight.
11. A foodstuff as claimed in claim 10 in which the amount of sorbitol is from about 0.3 to about 1.2% by weight.
12. A foodstuff as claimed in claims 10 or claim 11 characterised by the fact that it has a total nitrogenous material content of less than 15% and a sorbitol content between about 0.1% and about 2% by weight.
13. A foodstuff as claimed in claim 12 in which the total nitrogenous material content is from about 9% to about 13% by weight.
14. A foodstuff as claimed in claim 10 substantially as herein described with reference to the Examples.

Applicant	: Cindie M. Luhman	
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EXHIBIT M

of

DECLARATION UNDER 35 U.S.C. § 132

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[News, Profiles, Stocks and More about this company](#)Published / Filed: **Feb. 15, 1980 / March 15, 1977**Application Number: **FR1977007707602**IPC Code: **A23K_1/16;**ECLA Code: **None**Priority Number: **March 19, 1976 FI1976000760746**

Family:

PDF	Publication	Pub. Date	Filed	Title
	SE0426434C	May 11, 1983	March 15, 1977	FODERTILLEGG FOR IDISSLARI
	SE0426434B	Jan. 24, 1983	March 15, 1977	FODERTILLSATS FOR IDISSLAR
	FR2344233B3	Feb. 15, 1980	March 15, 1977	
	FR2344233A1	Oct. 14, 1977	March 15, 1977	ADDITIF DU FOURRAGE POUR RUMINANTS
	FI0053394C	June 7, 1983	March 19, 1976	FODERTILLSATS FOER FOERBAETTRING AV ENERGIBALANSEN HOS IDISSL GENOM FOERHOEJNING AV BLODSOCKERHALTEN
	DK0146192C	Dec. 27, 1983	March 18, 1977	DROEVTYGGERFODERTILSKU
	DK0146192B	July 25, 1983	March 18, 1977	DROEVTYGGERFODERTILSKU
	DE2710930C2	Sept. 27, 1990	March 12, 1977	VERWENDUNG 5- ODER 6-WER ZUCKERALKOHOLE

8 family members shown above

Other Abstract Info:

CHEMABS 088(01)005045V



Applicant	: Cindie M. Luhman	
Serial No.	: 09/239,873	
Filed	: January 29, 1999	Group Art Unit: 1616
For	: METHOD AND COMPOSITION FOR ENHANCING MILK COMPONENT CONCENTRATIONS	Examiner: N. Levy
Docket No.	: LL11.12-0040	

EXHIBIT N

of

DECLARATION UNDER 35 U.S.C. § 132

Examined Finnish Patent Application No. FI0053394B



[B] (11) KUULUTUSJULKAISU 53394
UTLÄGGNINGSSKRIFT

(45)

(51) Kv.Ik.³/Int.Cl.³ A 23 K 1/16

SUOMI—FINLAND

(FI)

**Patentti- ja rekisterihallitus
Patent- och registerstyrelsen**

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(32)(33)(31) Pyydetty etuoikeus — Begärd prioritet	

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(54) Rehun lisäaine märehitjän energiatasapainon parantamiseksi veren sokeri-
pitoisuutta kohottamalla - Fodertillsats för förbättring av energibalansen
hos idisslare genom förhöjning av blodsockerhalten

Keksinnön kohteena on märehitjööiden ruokinnassa käytettävä rehun lisäaine, joka vaikuttaa erittäin edullisesti lehmien maitodontuotantoon, tuotantoa lisäävästi ja rasvapitoisuuden vaihtelu- ja pienentävästi.

Elimistö tarvitsee aineenvaihduntaansa helppoliukoisia hiilihydraatteja, ennen kaikkea erilaisia sokereita, joista glukoosi on merkittävin, Yksimahaisilla isomolekyylliset hiilihydraatit, kuten tärkkelys, pilkkoutuvat sokeriksi ruoansulatusnesteiden ansiosta suolistossa. Märehitjööillä sama pilkkoutuminen tapahtuu jo etumahoissa, ennen kaikkea pötsissä, mikrobien toimesta. Nämä sekä ruokinnan mukana olevat sokerit käytetään mikrobien elintointojen energiana. Se muuttuu siis mikrobimassaksi, eikä siis suoranaaisesti kohota elimistön sokerin saantia.

Eläin, myös märehitjä, tarvitsee sokeria lähinnä maksan ja maitorauhasten toimintaan. Glukoosi muuttuu lehmän utareissa maitosokeriksi ja erittyy eläimestä maidon mukana. Lehmällä, jonka päivätuotos on 30 kg maitoa, tämä merkitsee n. 1500 g:n glukoosimäärää, joka poistuu elimistöstä päivittäin. Muut tarpeet huomioon

ottaen on arvioitu, että keskikokoisen lehmän sokerin tarve huipputuotannon vaiheessa olisi n. 2000 g/pv. Maksan tehtävänä on hankkia tämä sokerimäärä. Koska sokeria, erikoisesti glukosia, ei siirry merkittäviä määriä ohi pötsin, on märehtijöillä maksassa muodostettava glukosia. Tärkeitä lähteitä ovat pötsissä oleva propionihappo sekä elimistön suorittama glukosin muodostus valkuaisaineista ja maitohaposta. Kuitenkin on arvioitu, että 30 kg päivässä lypsävällä eläimellä sokerivajaus muodostuu n. 700 g:ksi. Tätä vajasta eläin pyrkii täyttämään hajoittamalla elimistön rasvoja. Tällöin syntyy glyserolia, joka muuttuu glukosiksi, mutta myös rasvahappoja, jotka puolestaan hajaantuessaan muodostavat asetonirunkoja. Eläin voi sairastua asetonitautiin, jos rasvojen hajoantuminen on liian runsasta.

Eläimen glukosin saannin turvaaminen vähentää rasvakudoksen hajoittamisen nopeutta, joka ei yleensä kuitenkaan riitä turvaamaan veren glukosipitoisuutta.

Utareiden glukosipitoisuudella on keskeinen asema maitomäärän säätelyssä. Jos glukosia on utarekudoksessa paljon, se imee osmoottisesti soluihinsa nestettä verestä osmoottisen paineen taasaamiseksi. Maitomäärä siis kasvaa. Jos sokeria on vähän tapahtuu päinvastainen ilmiö.

Koska nykyaikaiset korkeatuottoisiksi jalostetut märehtijät eivät läheskään aina pysty huolehtimaan glukosin tarpeestaan, on asian korjaamiseksi turvauduttu ruokinnallisiin apukeinoihin. Märehtijöille on annettu ruokinnassa glukogeenisiä lisäaineita, jotka eivät hajoa pötsissä vaan kulkeutuvat hajoamatta jäklimahoihin ja sieltä maksaan, jossa muuttuvat sokereiksi. Toinen tapa on pyrkiä kohottamaan pötsin propionihappopitoisuutta, koska propionihappo muuttuu maksassa tehokkaaksi glukosiksi. Tähän tarkoitukseen on tunnetusti käytetty seuraavia aineita: propyleeniglykoli, glyseroli, erilaiset propionaatit, jopa propionihappo.

Yllättäen olemme nyt keksinnön mukaisesti todenneet, että märehtijän rehuun lisättävä, suuren sokerialkoholipitoisuuden omaava lisäaine, jonka koostumus esiintyy edullisena koivupuuperustaisen ksylitolituotannon sivutuotteena syntyvässä erilaisten sokerialkoholien seoksessa, parantaa märehtijöiden glukositasetta oleellisesti, koska se käyttäytyy samantapaisesti kuin ym. glukogeeniset aineet. Lisäaineen tunnusmerkit ilmenevät oheisista patenttivaatimuksista.

Suositteltava on koostumus, jossa on ksylitolia, arabitolia, dulsitolia ja ramnitolia.

Keksinnön kohteena olevaa tuotetta kutsumme tässä sokerialkoholiseokseksi. Se on kellertävän ruskea makean makuinen neste, jonka ominaispaino 50%:sena vesiliuoksena on huoneenlämmössä n. 1,22. Sen kaloriarvo ja makeusaste ovat samat kuin glukosilla. Kyseisen tuotteen eräs edullinen koostumus on kuiva-aineesta laskettuna suunnilleen seuraava:

ksylitoli	15-25 %
arabitol	20-35 %
mannitoli	15-25 %
sorbitoli	5-15 %
dulsitoli	5-15 %
ramnitoli	5-10 %
Muut	2-5 %
hajoamistuotteet	2-5 %

Kirjallisuustietojen perusteella on tunnettua, että esim. ksylitoli stimuloi maksan toimintaa ja lisää soluaktiiviteettia. On myöskin tunnettua, että suun mikrobit eivät pysty käyttämään ksylitolia energian lähteenä.

Keksintömme perustuu kuitenkin aikaisemmin tuntemattomaan havaintoon, että sokerialkoholit säilyvät riittävän pitkän ajan hajaantumatta myös pötsin olosuhteissa, mikä ilmenee esimerkissä 1 annetuista koetuloksista.

Esimerkki 1:

Esimerkissä 1 on tutkittu yksittäisten sokerialkoholien hajaantumista pötsinesteessä in vitro.

Sokerialkoholien säilyminen pötsinesteessä (% lisätystä määrästä)

Inkubointiaika	2 t	4 t	8 t	24 t	48 t
Ksylitoli	91,2	89,7	88,4	83,4	33,8
Arabitol	86,8	93,0	88,3	87,0	55,9
Mannitoli	88,9	88,1	80,5	1,4	0,4
Dulsitoli	92,9	94,3	85,8	61,2	7,0
Sorbitoli	91,3	88,3	79,6	11,1	0,2

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Todetaan, että kaikki sokerialkoholit säilyvät hajaantumattomina lähes täydellisesti 8 t:n ajan, ksylitoli, arabitoli ja dulsitoli jopa 24 t ja arabitoli ja ksylitoli osittain tätäkin kauemmin. Tavallinen sokeri hajoaa jopa alle 2 tunnissa.

Esimerkki 2:

Tässä esimerkissä on tutkittu sokerialkoholiseoksen käyttäytymistä pötsinesteessä ja on todettu että sen säilyminen pötsinesteessä on samansuuntainen yksittäisten sokerialkoholien säilymisen kanssa. Käytetyn sokerialkoholiseoksen, joka oli koivusokerivalmistuksen sivutuote, koostumus oli

Ksylitoli	18	paino-%
Arabitoli	24	"-
Mannitoli	18	"-
Sorbitoli	9	"-
Galaksitoli	7	"-
Ramnitoli	7	"-
Pelkistäviä sokereita	7	"-
Muut polyolit	10	"-

joskin. ksylitolivalmistusprosessin talteenotto on parantunut ja koostumus vaihtelee uusissa laitoksissa seuraavissa rajoissa

Ksylitoli	10	± 4	paino-%
Arabitoli	15	± 6	"-
Mannitoli	16	± 3	"-
Sorbitoli	10	± 2	"-

Koetulos:

Inkubointiaika	2 t	4 t	8 t	24 t	48 t
Jäljellä % lisä-					
tystä määrästä	92,6	86,7	87,6	55,2	23,7

Koska nesteen viipymä pötsissä on vain muutama tunti, ehtii sokerialkoholi kulkeutua jälkimahoihin ennen kuin hajaantuminen pötsissä tapahtuisi.

Läpäistyään pötsin sokerialkoholit käyttäytyvät normaalin sokeriaineenvaihdunnan tavoin, joka näkyy esim. veren glukoosipitoisuuden kohoamisena, kuten selviää esimerkistä 3.

Esimerkki 3:

Veren glukoosipitoisuuden vaihtelu

Seurantavaihe

päiviä tutk.al.	0	10	13	15	17	18	19	21	25
Ei sokerialkoholia									
					Sokerialkoholia 2 x 125 g k.a.		2 x 250 g k.a.		
glukoosia m.mol./l	3,4	3,1	3,0	2,5	2,7	3,1	3,2	3,2	3,5

Normaaliruokinnalla koe-eläimen glukoosiarvo aleni jatkuvasti ollen ennen koeruokinnan aloittamista jo alle 3,0 m.mol./l. Sokerialkoholien (esim. 2 seos) annostus 2 x 125 g k.a. aloitettiin seurannan seitsemäntenätoista päivänä. Veren glukoosiarvot alkoivat kohota jo samana päivänä. Viikon kestäneen koeruokinnan kestäessä glukoosimäärä nousi seurannan alkamisajankohdan arvoon. Eläimen yleiskunto palasi ulkonaisesti arvostellen normaalisti.

Tutkittavalla koe-eläimellä maidon rasva-% vaihteli ennen sokerialkoholiseoksen annostamista lähes 2 prosenttiyksikköä päivittäin ollen keskimäärin 4,5. Koeruokinnan aloittamisen jälkeen rasva-% aleni kahden päivän kuluttua sille tasolle, joka eläimelle oli ominaista eli alle 4,0 %:n. Samanaikaisesti päivittäinen vaihtelu pieneni merkityksettömäksi.

Esimerkin 3 koe-eläimen energia- ja ennen kaikkea sokeriaineenvaihdunta voidaan todeta siinä määrin häiriytyneeksi, että asetonitaudin eli ketoosin ilmeneminen olisi ollut mahdollista, ellei koeruokintaa olisi aloitettu ja eläimen sokeriaineenvaihdunta palautettu normaaliin tilaansa.

Vaikuttamalla pötsin toimintaan siten, että propionihapon tuotanto lisääntyy etikkahapon ja voi-hapon kustannuksella, voidaan märehtijän sokeriaineenvaihduntaa parantaa, koska propionihappo muuttuu elimistössä erittäin tehokkaasti glukoosiksi.

Esimerkki 4 valaisee sokerialkoholiseoksen vaikutusta pötsin rasvahappotuotantoon. Etikkahapon osuuden lisääntyminen merkitsee energiahukkaa, koska sen edelleen metaboloituessa syntyy hiilidioksidi, joka poistuu käyttökelvottomana elimistöstä. Sen sijaan propionihappo muuttuu kokonaisuudessaan glukoosiksi.

Esimerkki 4:

Fistelien kautta otetuilla pötsinäytteillä seurattiin etikkahapon (E) ja propionihapon (Pr) osuuksien muuttumista pötsinesteessä.

Seuratavaiheessa E:n osuus oli nouseva ja Pr:n laskeva. Koeruokinnan alettua suhde alkoi muuttua toiseen suuntaan. Ulkonaisesti arvostel-
len eläin piristyi silminnähden.

Seurantavaihe	1	2	3	4	5	6
E, %	51	60	62	57	51	52
Pr, %	28	26	25	27	28	29

Seurantavaiheet 1-3 vastasivat talviruokintaa perusrehulla, jolloin ruokinnan aikana ilmeni oireita sokeriaineenvaihdunnan epätasapainon kehittymisestä, vaiheessa 4 on lehmälle annosteltu sokerialkoholi-
seosta (esim. 2 seos) 2 x 125 g k.a./pv., vaiheessa 5 on annos 2 x 250 g k.a./pv. ja vaiheessa 6 2 x 200 g k.a./pv. sokerialkoholia kui-
vana laskettuna päivässä eläintä kohti.

Voidaan todeta, että pötsin propionihapon tuotanto lisääntyi yli 100 g:lla päivässä sokerialkoholiseoksen ansiosta. Suurin osa sokerialkoholiseoksesta kuitenkin kulkeutuu hajoamatta ohi pötsin.

Esimerkki 5:

Lehmälle, joka oli jo ohittanut huipputuotosvaiheen poikimisen jälkeen, annettiin sokerialkoholiseospitoista (esim. 2) liuosta 0,4 l päivässä. Maitomäärä alkoi selvästi kohota, niin että se koeruokin-
nan aikana, joka kesti n. 4 viikkoa, herui 0,1 kg päivää kohti. Esi-
merkkieläin oli jo ohittanut tuotantohuippunsa koeruokinnan alkaessa, jolloin energiantarve glukooseina jo oli laskenut siitä, mitä se oli ollut suurimmillaan. Siitä huolimatta eläin lisäsi tuotantoaan. He-
rumisvaiheessa olevalla eläimellä ei vastaavaa koetta voida aikaansaa-
da, koska ei voida sanoa mikä osa tuotoksen noususta on tuotosvaiheen ja mikä osa lisärehun aiheuttamaa.

Lisäaineseoksen kantaja-ainekomponenttina taikka rehuainekompo-
nenttina käytetään edullisesti melassin liuoksia. Seokseen voidaan myös lisätä sinänsä tunnettuja glukogeenisia aineita, kuten esimerkik-
si propyleeniglykolia, jolloin keksinnön mukaista sokerialkoholiseoksen osuutta voidaan vastaavasti pienentää. Sokerialkoholiseoksen osuus on kuitenkin aina vähintään 40 paino-% seoksen kuivapainosta laskettuna.

Keksintömme mukaista rehun lisäaineseosta voidaan käyttää liuok-
sena, jolloin se on pakattu sopiviin säiliöihin ja annostellaan suo-
raan ruokinnan yhteydessä määrin 100-1000 g kuiva-aineksi laskettu-
na (sokerialkoholia 40-500 g) päivässä eläintä kohti. Lisäaine voi-
daan myös kuivata ja lisätä esim. muihin tehdasvalmisteisiin rehuihin tai käyttää sellaisenaan kuivana säkkitavarana.

Patenttivaatimukset:

1. Märehtijän rehun lisääaine, joka erityisesti kohottaa lehmän veren sokeripitoisuutta ja lisää maidontuotantoa, t u n n e t t u siitä, että se sisältää sinänsä tunnetun rehuainekomponentin ja mahdollisten tunnettujen glukogeeniaineiden lisäksi 40-85 % seoksen kuivapainosta laskettuna yhtä tai useampaa viisi- ja/tai kuusiarvoista sokerialkoholia.

2. Patenttivaatimuksen 1 mukainen rehun lisääaine, t u n n e t t u siitä, että se sokerialkoholeina sisältää ksylitolia, arabitolia, dulcitolia ja ramnitolia.

3. Patenttivaatimuksen 1 mukainen rehun lisääaine, t u n n e t t u siitä, että sen sokerialkoholijae on ksylitolia koivupuusta valmistettaessa syntyvä sivutuote.

4. Patenttivaatimuksen 3 mukainen rehun lisääaine, t u n n e t t u siitä, että sokerialkoholijakeen koostumus on kuiva-aineesta laskettuna ksylitolia 15-25 %, arabitolia 20-35 %, mannitolia 15-25 %, sorbitolia 5-15 %, dulcitolia 5-10 % ja ramnitolia 5-10 %.

5. Patenttivaatimuksen 1 mukainen rehun lisääaine, t u n n e t t u siitä, että lisääaineseosta käytetään nesteinä tai kiinteäksi kuivattuna, edullisesti annoksena joka on 40-500 g sokerialkoholeja kuivana laskettuna eläintä kohti päivässä.

6. Patenttivaatimuksen 1 mukainen rehun lisääaine, t u n n e t t u siitä, että rehuainekomponenttina käytetään melassia.

7. Patenttivaatimuksen 1 mukainen rehun lisääaine, t u n n e t t u siitä, että sinänsä tunnettuna glukogeeniaineena käytetään propyleeniglykolia.

Patentkrav:

1. Fodertillsats för idisslare som speciellt ökar blodets sockerhalt och mjölkproduktionen hos kor, k ä n n e t e c k n a d därav, att den förutom en i och för sig känd foderkomponent och eventuella kända glukogena ämnen innehåller 40-85 % av en eller flera fem- och/eller sexvärda sockeralkoholer beräknat av blandningens torrsvikt.

2. Fodertillsats enligt patentkravet 1, k ä n n e t e c k n a d därav, att den såsom sockeralkoholer innehåller xylitol, arabitol, dulcitol och ramnitol.

3. Fodertillsats enligt patentkravet 1, k ä n n e t e c k n a d därav, att dess sockeralkoholfraktion är en biprodukt vid xylitolframställningen ur björkved.

4. Fodertillsats enligt patentkravet 3, k ä n n e t e c k n a d därav, att sockeralkoholfraktionens sammansättning är, på torrsustansen beräknat, xylitol 15-25 %, arabitol 20-35 %, mannitol 15-25 %, sorbitol 5-15 %, dulcitol 5-10 % och ramnitol 5-10 %.

5. Fodertillsats enligt patentkravet 1, k ä n n e t e c k n a d därav, att tillsatsblandningen användes i form av en vätska eller i torkad form, företrädesvis i en daglig dos av 40-500 g sockeralkohol, beräknat som torrsustans, per djur.

6. Fodertillsats enligt patentkravet 1, k ä n n e t e c k n a d därav, att såsom foderkomponent användes melass.

7. Fodertillsats enligt patentkravet 1, k ä n n e t e c k n a d därav, att såsom i och för sig känt glukogent ämne användes propylenglykol.

Viitejulkaisuja-Anförda publikationer

Patenttijulkaisuja:-Patentskrifter: Sveitsi-Schweiz(CH) 497 850 (A 23 k 1/00).

Applicant	: Cindie M. Luhman	
Serial No.	: 09/239,873	
Filed	: January 29, 1999	Group Art Unit: 1616
For	: METHOD AND COMPOSITION FOR ENHANCING MILK COMPONENT CONCENTRATIONS	Examiner: N. Levy
Docket No.	: LL11.12-0040	

EXHIBIT O

of

DECLARATION UNDER 35 U.S.C. § 132

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ⓘ Title: **FI0053394B:**
 ⓘ Country: **FI Finland**
 ⓘ Kind: **B Examined Application** ⁱ (See also: [FI0053394C](#))
 ⓘ Inventor: **None** [No Image](#)
 ⓘ Assignee: **MERENSALMI MATTI, Finland**
 [News, Profiles, Stocks and More about this company](#)
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ⓘ Family:

PDF	Publication	Pub. Date	Filed	Title
	US4127676	Nov. 28, 1978	March 17, 1977	Fodder additive for ruminants
	SU0626678D	Sept. 30, 1978	March 18, 1977	FEED ADDITION
	SE7702937A	Sept. 20, 1977	March 15, 1977	FODERTILLSATS FOR IDISSLA
	SE0426434C	May 11, 1983	March 15, 1977	FODERTILLEGG FOR IDISSLA
	SE0426434B	Jan. 24, 1983	March 15, 1977	FODERTILLSATS FOR IDISSLA
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	IE0045123B	June 30, 1982	March 15, 1977	IMPROVEMENTS IN OR RELAT FOODSTUFFS FOR RUMINANT
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	FR2344233B3	Feb. 15, 1980	March 15, 1977	
	FR2344233A1	Oct. 14, 1977	March 15, 1977	ADDITIF DU FOURRAGE POUR RUMINANTS
	FI0760746A	Sept. 20, 1977	March 19, 1976	
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	FI0053394C	June 7, 1983	March 19, 1976	FOERBAETTRING AV ENERGIBALANSEN HOS IDISSI GENOM FOERHOEJNING AV BLODSOCKERHALTEN
	FI0053394B	Jan. 31, 1978	March 19, 1976	
	DK0146192C	Dec. 27, 1983	March 18, 1977	DROEVTYGGERFODERTILSKU
	DK0146192B	July 25, 1983	March 18, 1977	DROEVTYGGERFODERTILSKU
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	DE2710930C2	Sept. 27, 1990	March 12, 1977	VERWENDUNG 5- ODER 6-WEI ZUCKERALKOHOLE
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	DD0129613Z	Feb. 1, 1978	March 18, 1977	FUTTERZUSAETZE FUER WIEDERKAEUER
	CS0191333P	June 29, 1979	March 15, 1977	ADMIXTURE IN THE FODDER F THE COWS
	CA1101263A1	May 19, 1981	March 16, 1977	FODDER ADDITIVE FOR RUMIN
25 family members shown above				

Other Abstract
Info:

CHEMABS 088(01)005045V



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